INFECTION AND IMMUNITY, Dec. 1993, p. 5345–5350
0019-9567/93/125345-06$02.00/0

Vol. 61, No. 12

Comparison of Naturally Acquired and Vaccine-Induced Antibodies to *Haemophilus influenzae* Type b Capsular Polysaccharide

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Received 21 May 1993/Returned for modification 7 July 1993/Accepted 21 September 1993

The objective of this study was to assess qualitative differences in the types of *Haemophilus influenzae* type B (Hib) capsular polysaccharide (polyribosylribitol phosphate [PRP]) antibodies induced in children 15 to 27 months of age by (i) natural exposure, (ii) PRP vaccine, and by (iii) PRP-diphtheria toxoid conjugate vaccine, (iv) PRP-group B *Neisseria meningitidis* outer membrane vesicle conjugate vaccine, and (v) *Haemophilus* type B oligosaccharide conjugate vaccine (HbOC). The highest levels of total Hib-PRP antibody measured by radioimmunoassay and immunoglobulin G (IgG) measured by enzyme-linked immunosorbent assay were seen after HbOC immunization. IgG1 Hib-PRP antibodies predominated in all groups, and there were no differences between the groups in the proportion of IgG and IgA Hib-PRP antibodies. However, the proportions of IgM differed significantly by group. The highest proportions of IgM occurred in naturally acquired antibody and after PRP vaccine, and the lowest proportion occurred after HbOC vaccine. IgG light-chain VκII type α PRP antibody was present in all groups, and the level correlated with the total IgG Hib-PRP antibody level. Therefore, HbOC induced the highest concentrations of VκII type α PRP antibody, and the naturally acquired antibody group had the lowest levels. IgG light-chain VκIII antibody levels were also highest in the HbOC group, but there was no correlation between VκIII antibody levels and total amount of IgG Hib-PRP antibody. These data demonstrate qualitative differences in the antibody repertoires induced by natural exposure, the Hib-PRP vaccine, and each of the different Hib conjugate vaccines. We doubt that there are major differences in the protection afforded by these different antibody repertoires, because these differences do not appear to correlate with differences in protective efficacy in older children.

Several different vaccines have been developed and licensed for the prevention of *Haemophilus influenzae* type b (Hib) disease. The first vaccine, available in 1985, was a purified Hib capsular polysaccharide polyribosylribitol phosphate (PRP) vaccine. PRP had limited immunogenicity and protective efficacy in children (15, 36, 37, 41). To enhance immunogenicity to the Hib-PRP, several Hib conjugate vaccines were developed. Hib conjugate vaccines differ in composition and structure by (i) the type of protein carrier, (ii) the molecular size and ratios of the Hib-PRP, and (iii) the type of linkage between the Hib-PRP and protein carrier. The first conjugate vaccine licensed was PRP-diphtheria toxoid conjugate vaccine (PRP-D), which has medium lengths of Hib-PRP linked via a six-carbon spacer to diphtheria toxoid. Although more immunogenic than PRP, the vaccine had limited immunogenicity in young infants (26, 51, 59). To improve immune responses in younger children, PRP-group B *Neisseria meningitidis* outer membrane vesicle conjugate vaccine (PRP-OMP) was developed, which links outer membrane vesicles from group B *N. meningitidis* to medium lengths of Hib-PRP. PRP-OMP induces high levels of antibody (Ab) in young infants after a single immunization, but subsequent immunizations do not appreciably enhance Ab levels (1, 42, 60). Nevertheless, PRP-OMP has been shown to be safe and effective in preventing Hib disease (42). A third conjugate vaccine, Hib oligosaccharide conjugate vaccine (HbOC), uses short Hib capsular oligosaccharides linked directly to a nontoxic variant of diphtheria toxin (CRM_{197}). HbOC provides lasting protection to infants after a series of two or three doses (27, 60), and it is the most widely used Hib conjugate vaccine in the United States.

The quantitative differences in Ab levels induced by these different Hib-PRP vaccines have been described previously (19, 51, 60). In adults and children, the Ab response to Hib-PRP is clonally restricted, unlike the Ab responses induced by most protein antigens (20, 25, 54). Functional differences in the isotype (immunoglobulin G [IgG], IgA, and IgM) and IgG subclass Abs have been observed in subjects immunized with different Hib vaccines (4, 12, 23, 34). Furthermore, the specificity of the IgG light-chain variable region of the Hib-PRP Abs has been characterized by idiotype (28) and amino acid sequence analysis (48–50). Recently, monoclonal Abs (MAbs) to specific IgG light-chain variable region sequences have been used to detect specific Hib Ab repertoires (50). Although functional and protective differences in the Abs of different Hib-PRP Ab isotypes have been reported (46), the functional or protective differences in IgG subclass or light-chain variable region Abs have not been defined.

We evaluated qualitative differences in Hib Ab repertoires induced by natural Hib exposure, PRP vaccine, and three Hib conjugate vaccines (PRP-D, PRP-OMP, and HbOC) in children 15 to 27 months of age. This age group was selected

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TABLE 1. Study design

Group (n)	Vaccine	Age (months) at immunization	Geometric mean titer (μg/ml)[a]	Titer range[a]
1 (24)	None (natural immunity)	16–24 (not vaccinated)	0.97	0.38–6.36
2 (17)	PRP	18–27	0.86[b]	0.21–11.50
3 (22)	PRP-D	16–24	1.43[b]	0.38–17.50
4 (24)	PRP-OMP	15–18	5.86[b]	0.43–34.00
5 (20)	HbOC	15–24	16.64[b]	2.70–240.0

[a] Total Hib-PRP Ab measured by RIA.
[b] Antibody titers 1 month past immunization.

because an immune response occurs with natural exposure and only one dose of each vaccine is required to induce Abs. Our objectives were to determine whether important qualitative differences in the types of Abs exist with different types of Hib-PRP exposure and which differences might be relevant in the development of other PRP conjugate vaccines.

MATERIALS AND METHODS

Study design. Table 1 outlines the study design for the five study groups: group 1, naturally acquired Ab; group 2, PRP; group 3, PRP-D; group 4, PRP-OMP; group 5, HbOC. All subjects were randomly selected from prior studies of PRP, PRP-D, and PRP-OMP vaccines in the Southern California Kaiser-Permanente Program. Serum samples from the HbOC group were kindly provided by Praxis Biologics. Except for the naturally acquired Ab group, all children were immunized with a single dose of Hib vaccine between 15 and 27 months of age. None of the children in the naturally acquired Ab group received any Hib vaccine prior to the study. Serum samples were obtained before and 1 month after immunization. The naturally acquired Ab group was randomly selected from individuals with a preimmunization Hib Ab titer greater than 0.15 μg/ml by radioimmunoassay (RIA). The age distributions for all of the groups are similar.

To evaluate vaccine-induced Abs, subjects in the four vaccine groups were selected for this study if their preimmunization titers were <0.15 μg/ml by RIA. Subjects who had a poor Ab response after immunization, as defined by an Ab level of <0.15 μg/ml by RIA, were excluded. Therefore, samples selected for each vaccine group had Hib-PRP Ab levels of >0.15 μg/ml by RIA, a level detected by our assays and considered protective (6, 24, 40, 45).

RIA. Total Hib-PRP Ab was assayed by a modified Farr RIA with intrinsically labeled PRP antigen. Details of this assay and the serum standards used have been previously described (5). The lower limit of sensitivity of this assay was 0.025 μg/ml, and for statistical analysis this value was assigned to all measurements <0.025 μg/ml.

IEF. Hib-PRP Abs in selected serum samples were separated and analyzed by isoelectric focusing (IEF) in agarose gels and visualized by labeling with ^{125}I-Tyr-Hib-PS and autoradiography. Modification of the IEF method described by Shackelford et al. was used (53). Twenty to 55 μl of serum was applied with an application mask and allowed to enter the gel at 10 mA of constant current for 20 min. Dried gels were exposed to film (Cronex 4L) for 24 to 48 h to visualize Hib-PRP Ab spectrotypes.

Isotype-specific PRP Ab ELISA. IgG, IgA, and IgM Hib-PRP Abs were quantitated by enzyme-linked immunosorbent assay (ELISA). Wells of E.I.A./R.I.A. flatplates (Costar, lot 3591) were coated with 0.1 ml of 1.0 μg of HbO-HA antigen (short-chained Hib capsular oligosaccharide conjugated to human serum albumin) (38) in phosphate-buffered saline (PBS) at pH 7.1 for 90 min at 37°C. The HbO-HA was kindly provided by Dace Madore (Praxis Biologics). After washing the wells, serum samples were incubated at various dilutions for 1 h at room temperature in PBS with 0.3% Tween 20 and 0.01 M EDTA at pH 7.2. The Office of Biologic Research and Review reference serum (U.S. standard for human Hib-PRP Abs) was incubated at room temperature on the same plate in PBS-Tween 20-EDTA at serial dilutions beginning at 0.305 μg/ml for IgG, 0.056 μg/ml for IgA, and 0.035 μg/ml for the IgM assays (Food and Drug Administration quantitation values). Specific IgG (0.1 μg/ml), IgM (0.2 μg/ml), or IgA (0.2 μg/ml) goat anti-human alkaline phosphatase (Tago) was added as the conjugate for 1 h at room temperature. The plates were washed, and disodium *p*-nitrophenyl phosphate in diethanolamine at pH 9.8 was used as the substrate at 1 μg/ml. The plates were read in an ELISA reader (Titertek Multiskan) at a wavelength of 405 nm. The lower limits of sensitivity for the IgG, IgA, and IgM Hib-PRP Ab assays were 0.005, 0.004, and 0.002 μg/ml, respectively. For statistical analysis, these values were assigned to all measurements below these concentrations.

IgG subclass PRP Ab ELISA. The IgG1 and IgG2 Hib-PRP subclass Abs were measured by a sandwich ELISA. Wells of E.I.A./R.I.A. flatplates (Costar) were coated with 0.1 ml of 1.0-μg/ml HbO-HA antigen in PBS at pH 7.1 overnight at room temperature. After washing, the plates were blocked with 1% bovine serum albumin (BSA) in PBS–0.1% Tween for 30 min at room temperature. The serum samples were incubated at various dilutions overnight at room temperature in PBS–0.1% Tween 20–BSA (pH 7.2). The Office of Biologic Research and Review reference serum was added to the plate at serial dilutions beginning at 0.54 μg/ml for IgG1 and 0.26 μg/ml for IgG2 (Food and Drug Administration assigned values). The human MAbs to the Hib-PRP C-G1 (0.22 μg/ml), for IgG1, and B-G2 (0.18 μg/ml), for IgG2, were added as positive controls for the assay (49). The plates were washed, and the MAbs HG11, for IgG1, and HP6016, for IgG2, were added at a concentration of 2.0 μg/ml in PBS–0.1% Tween 20–BSA and incubated overnight at room temperature. The HG11 (mouse anti-human IgG1 MAb) and HP6016 (mouse anti-human IgG2 MAb) have been previously described (58). The plates were washed, and goat anti-mouse IgG alkaline phosphatase (Southern Biotechnology) was added at a dilution of 0.2 μg/ml overnight at room temperature. After washing, disodium *p*-nitrophenyl phosphate was added and the plates were developed as in the previous ELISA. The lower limits of sensitivity for the IgG1 and IgG2 Hib-PRP Ab assays were 0.017 and 0.004 μg/ml, respectively. For statistical analysis, these values were assigned to all measurements below these concentrations. IgG3 and IgG4 Hib-PRP Abs were not assayed in this study because of the low Ab levels elicited by polysaccharide vaccines for these IgG subclasses (21, 23, 30, 32).

VκII type α PRP and VκIII Hib-PRP Abs. VκII type α PRP and VκIII Ab levels were determined by a sandwich ELISA that has been previously described in detail (13). VκII type α PRP Ab was translated from the VκIIA2 gene (50). Briefly, the wells of an Immulon 2 plate (Dynatech) were coated with 0.1 ml of Hib-PRP–poly-L-lysine (13) at 1.0 μg/ml. After washing, the plates were blocked with 1% BSA in PBS at room temperature. Samples from immunized subjects were

TABLE 2. Levels of Hib-PRP Abs by isotype and subclass

Group	(n)	Geometric mean titer in μg/ml (ratio [%] to IgG + IgA + IgM)				
		IgG	IgA	IgM	IgG1	IgG2
Natural Ab	(24)	0.30 (61)	0.02 (4)	0.17 (35)	0.07	0.01
PRP	(17)	0.26 (51)	0.08 (16)	0.17 (33)	0.19	0.09
PRP-D	(22)	0.41 (71)	0.05 (9)	0.12 (21)	0.29	0.07
PRP-OMP	(24)	2.44 (78)	0.13 (4)	0.52 (17)	1.78	0.15
HbOC	(20)	11.90 (81)	0.48 (4)	2.23 (15)	4.09	0.14

added at various dilutions and incubated at room temperature. Reference serum that contained pooled serum samples from three adult subjects that had assigned values of 37 U of VκII type α PRP Ab and 5 U of VκIII Ab per ml was added to the plate. With purified clonal Hib-PRP Abs, a preliminary study found that 1 U of the reference serum was standardized to approximately 1 μg of Hib-PRP Ab. Mouse MAb KB13 (13) was used for the VκII type α PRP assay, and mouse MAb B12 (29) was used for the VκIII assay. Goat anti-mouse Ig alkaline phosphatase Ab (13) was used as the conjugate and disodium *p*-nitrophenyl phosphate (Sigma) was used as the substrate at 1 μg/ml. The plates were read in an ELISA reader (Dynatech) at a wavelength of 405 nm. The lower limits of sensitivity of the VκII type α PRP and VκIII assays were 0.12 and 0.065 U/ml, respectively. For statistical analysis, these values were assigned to all measurements below these concentrations.

Statistical analysis. Statistical analyses were performed with the BMDP program. Probability values were computed for correlation with Fisher's exact test. The means were compared with Student's *t* test.

RESULTS

Total Hib-PRP Ab. The range and mean total Hib-PRP Ab levels for each group as measured by RIA are shown in Table 1. The highest Ab levels were in the group given HbOC. Lower levels were seen in the PRP-OMP and PRP-D groups. The naturally acquired Ab and PRP groups had the lowest total Ab levels. The difference in the titers between the groups is not an absolute indication of relative immunogenicity, because only samples with RIA titers of >0.15 μg/ml were selected. For each vaccine group, there was no correlation between the total Hib-PRP Ab concentration before immunization and the total Hib-PRP Ab concentration after immunization. The Hib Ab repertoires in the vaccine groups probably do not reflect prior priming, because only 14 of the 83 serum samples collected from subjects prior to immunization had detectable Hib-PRP Ab by RIA (>0.025 μg/ml but <0.150 μg/ml [7 of 17 in the PRP group, 1 of 22 in the PRP-D group, 4 of 24 in the PRP-OMP group, and only 2 of 20 in the HbOC group]).

IEF. IEF was initially performed to assess the diversity of the Hib-PRP Abs in each study group. When assessed by IEF, there were no major differences in the Hib-PRP Ab repertoires observed between the groups (data not shown). There were fewer than five Ab clonotype patterns observed in each serum sample.

Hib-PRP isotype and IgG subclass. Table 2 summarizes Hib-PRP Ab levels in each group for IgG, IgA, and IgM, as well as the IgG1 and IgG2 subclass (geometric mean titer in micrograms per milliliter). HbOC induced the highest IgG, IgA, and IgM PRP Ab levels. The proportion of IgG Hib-PRP Ab to total Hib-PRP Ab (ratio of IgG to IgG plus IgA plus IgM) was compared in the naturally acquired Ab group and in each of the Hib vaccine groups. No significant differences were found for the IgG ratios in these five groups. However, the lowest proportion of IgG and the highest proportion of IgM (ratio of IgM to IgG plus IgA plus IgM) were in the naturally acquired Ab and PRP groups. Similar but lower proportions of IgM were found in the PRP-D, PRP-OMP, and the HbOC groups. Compared with the total amount of Hib-PRP Ab present, all of the groups had very little IgA Hib Ab. Although the proportions of IgA Hib-PRP Ab between any of the groups were not significantly different, it is interesting that HbOC had the highest IgA Ab level. The total Hib-PRP Ab levels (RIA) did correlate with the IgG Hib-PRP levels (ELISA) for all of the vaccine groups ($P < 0.002$).

In all of the groups, the predominant IgG subclass was IgG1 Hib-PRP Ab (Table 2). The HbOC and the PRP-OMP groups had the highest level of IgG1 Hib-PRP Ab, followed by the PRP and PRP-D groups. The naturally acquired Ab group had the lowest IgG1 Hib-PRP Ab concentrations. The HbOC and PRP-OMP groups also had the highest level of IgG2 Hib-PRP Ab, followed by the PRP and PRP-D groups. Again, the naturally acquired Ab group had the lowest IgG2 Hib-PRP Ab level. However, the IgG2 Hib-PRP Ab concentrations were low for all the groups. The mean IgG Hib-PRP Ab concentration does not equal the sum of the IgG1 or IgG2 Hib-PRP Ab concentrations for each group because these ELISA assays have different methods and standards. However, the IgG Hib-PRP level did correlate with the IgG1 and IgG2 Hib-PRP Ab levels ($P < 0.02$ and $P < 0.03$, respectively).

VκII type α PRP and VκIII PRP Abs. In all of the groups, VκII type α PRP was the predominant IgG light-chain Ab (Table 3). Importantly, for the PRP, PRP-D, PRP-OMP, and HbOC groups, there is a strong correlation between VκII type α PRP Ab levels (units per milliliter) and the total IgG Hib-PRP Ab level ($P < 0.05$ for each group), with increasing levels of IgG Hib-PRP Ab correlating with increased levels of VκII type α PRP Ab. However, this association was not

TABLE 3. VκII type α PRP and VκIII specific Hib-PRP Ab levels

Group	VκII type α PRP Ab in U/ml (95% confidence interval)	% with detectable VκII type α PRP	Correlation of VκII type α PRP with IgG	VκIII Ab in U/ml (95% confidence interval)	% with detectable VκIII	Correlation of VκIII with IgG
Natural Ab	0.14 (0.12–0.28)	29 (6 of 21)	$r = 0.14$, $P = 0.53$	0.11 (0.07–0.22)	48 (10 of 21)	$r = 0.57$, $P = 0.008$
PRP	0.50 (0.15–1.69)	69 (9 of 13)	$r = 0.62$, $P = 0.03$	0.07 (0.07–0.11)	38 (5 of 13)	$r = 0.18$, $P = 0.06$
PRP-D	0.65 (0.38–1.10)	71 (15 of 27)	$r = 0.84$, $P < 0.001$	0.14 (0.10–0.20)	24 (5 of 21)	$r = 0.009$, $P = 0.97$
PRP-OMP	2.10 (1.24–3.58)	88 (21 of 24)	$r = 0.74$, $P < 0.001$	0.17 (0.12–0.25)	38 (9 of 24)	$r = 0.31$, $P = 0.14$
HbOC	10.38 (5.44–19.79)	100 (20 of 20)	$r = 0.68$, $P = 0.001$	0.68 (0.42–1.10)	100 (20 of 20)	$r = 0.11$, $P = 0.63$

seen in the naturally acquired Ab group, in which high levels of total IgG Hib-PRP Ab were not associated with higher VκII type α PRP Ab concentrations.

There was very little detectable VκIII antibody in the PRP, PRP-D, and PRP-OMP groups, but all of the serum samples in the HbOC group had measurable levels of VκIII Hib-PRP Ab. The VκIII Ab levels were greatest in the HbOC group and least in the PRP-D group. The naturally acquired Ab group had higher percentages of detectable VκIII Abs. In contrast to the relationship with the VκII type α PRP Abs, there was no correlation between VκIII Ab levels and total IgG Hib-PRP Ab in any of the study groups; increasing levels of IgG Hib-PRP Ab were not associated with an increase in VκIII Ab.

DISCUSSION

These and other data confirm that the Hib vaccines elicit quantitatively and qualitatively different Ab repertoires (19, 27, 30, 40, 60). The Hib vaccines that induce high levels of IgG Hib-PRP Ab would be the most desirable because there is evidence to suggest that high concentrations of IgG Hib-PRP Ab confer increased bactericidal and protective activity (7, 12, 14, 33, 34). In our study, HbOC was found to have induced the highest levels of IgG Hib-PRP Ab. This was followed by PRP-OMP and then PRP-D. The lowest levels of IgG Hib-PRP Ab levels were in the PRP and the naturally acquired Ab groups. This was expected, because PRP is a poor immunogen in children less than 2 years of age (15, 36, 51, 55) and children usually do not naturally develop natural immunity to PRP antigens until later in life (10, 37, 39). Even with invasive Hib disease, young children fail to develop Hib-PRP Abs after infection (22, 35).

The proportions of IgM Hib-PRP Ab were highest in the naturally acquired and PRP vaccine groups. Because Hib-PRP is a T-independent antigen (9, 56), the high proportion of IgM Hib-PRP Ab likely represents a less complete switch of B lymphocytes from IgM to other isotypes. The conjugate vaccines induced lower levels but similar proportions of IgM Hib-PRP Abs.

The importance of IgA for protection from Hib disease is not known. IgA Hib-PRP Ab is not bactericidal (46). The proportions of IgA Hib-PRP Abs in our serum samples were only slightly different for each group, with the highest IgA Hib-PRP Ab levels induced in the HbOC and PRP-OMP groups. Interestingly, the naturally acquired Ab group, which had Abs presumably induced by mucosal exposure, had very little IgA Hib-PRP Ab. However, we did not measure IgA levels in respiratory secretions and we cannot make any conclusion about the importance of IgA-specific Abs in the prevention of invasive Hib disease. There is IgG in mucosal secretions (8, 11), and this may be important for protection. Takala et al. reported a decrease in the oropharyngeal carriage of Hib in patients receiving Hib conjugate vaccines (57). This is in contrast to unconjugated PRP vaccines for which there is no apparent change in the carriage rate in vaccinated individuals (16, 18). Takala proposed that this difference may be due to higher concentrations of Hib-PRP Ab in serum via an enhanced serum Ab response leading to higher mucosal transudation of IgG PRP Ab. This enhanced Ab response (booster effect) is explained by the T-cell-dependent properties in the Hib conjugate vaccines (9, 56).

For all groups the predominant IgG subclass of Hib-PRP Abs was IgG1 (19, 23). After immunization of adults with Hib conjugate vaccines, there is a similar distribution of IgG1 and IgG2 Abs (30, 53). However, children do not begin to produce IgG2 until later in life and do not achieve adult levels of IgG2 until 8 to 10 years of age (31, 47, 52). This probably accounts for the IgG1 predominance in these sera from younger children. Although one study suggested that there is a difference between the bactericidal activity of the IgG1 and IgG2 Hib-PRP Abs (4), this difference was minor and was not confirmed in a more recent study (61). Amir et al. proposed that differences in the avidity of IgG subclass Hib-PRP Abs may be a more important factor because avidity correlates with bactericidal activity (3). Schlesinger et al. (43) have shown differences between the avidities of Hib-PRP Abs that were elicited by different Hib conjugate vaccines. They demonstrated that the HbOC vaccine elicited higher-avidity Abs and better bactericidal activity than Abs induced by the PRP-OMP. However, this in vitro difference may not be important, because there have been very few vaccine failures with PRP-OMP in children (42).

The Hib-PRP Ab response is restricted (49, 50, 53). The VκII type α PRP Ab is the predominant IgG light chain expressed in response to exposure to the Hib-PRP or conjugated vaccines (2, 48, 49). The level of VκII type α PRP Ab correlates with the total IgG Hib-PRP Ab concentration, and it is therefore difficult to distinguish between the functional differences of VκII type α PRP Abs and the amount of total IgG Ab. There was more variability between groups in the expression of VκIII Abs, and there was no association between total IgG Hib-PRP Ab and VκIII Ab concentration. Only in the naturally acquired Ab group were there more samples that contained detectable VκIII than there were samples that contained VκII type α PRP Abs. All of the serum samples assayed in the HbOC group had measurable levels of VκIII Ab, whereas this Ab was infrequent in the other groups. VκIII Hib-PRP Ab may cross-react with the *Escherichia coli* K100 capsular carbohydrate Ab (44, 49). Individuals with naturally acquired Hib-PRP Ab probably developed this early in life as an immune response to *E. coli* or other cross-reacting bacteria, and this may be beneficial for protection.

The most important unresolved issue with regard to Hib-PRP Ab repertoires is knowledge of potential differences in protection afforded by different types of Abs. This was not addressed directly by our study, but several conclusions can be inferred. There were no significant differences in the Ab repertoires between the study groups. Limited repertoires were seen in serum specimens with the lowest amount of total Hib-PRP Ab. Conversely, children with the highest Hib-PRP Ab concentrations had the greatest Ab diversity. Assessing differences by proportion of total Hib-PRP Ab, the following results were observed. (i) The naturally acquired Ab and PRP groups were similar, having the lowest titers of IgG Hib-PRP Ab, the greatest proportion of IgM Hib-PRP Ab, and the least amount of VκII type α PRP Abs. (ii) The HbOC group had the highest titers of IgG and IgA Hib-PRP Ab, the least IgM Hib-PRP Ab, and the most VκII type α PRP antibody. (iii) The PRP-D and PRP-OMP groups were between these two extremes. For all of the groups, the predominant IgG subclass was IgG1. The biologic importance of the different Hib Ab repertoires is not known. Assuming from published efficacy data from older children that HbOC, PRP-OMP, and PRP-D offer equivalent protection (1, 17, 42, 62), one might conclude that these differences in Ab repertoires are insignificant. However, we feel that protection studies of animals with purified Hib-PRP Abs are needed to confirm this conclusion. We are currently studying the repertoires of Hib-PRP Abs in Californian and Native

Alaskan infants immunized with PRP-D, PRP-T, and PRP-OMP to determine whether there are differences by age or ethnic group.

ACKNOWLEDGMENTS

This work was supported by NIH grants 5T32HD07245 and RO1 AI31580-02 and NIH contract N01-AI-15124.

We thank Pamela Moore for secretarial assistance.

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